Research paper

Variation in leaf and twig CO$_2$ flux as a function of plant size: a comparison of seedlings, saplings and trees

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Rates of tissue-level function have been hypothesized to decline as trees grow older and larger, but relevant evidence to assess such changes remains limited, especially across a wide range of sizes from saplings to large trees. We measured functional traits of leaves and twigs of three cold-temperate deciduous tree species in Minnesota, USA, to assess how these vary with tree height. Individuals ranging from 0.13 to 20 m in height were sampled in both relatively open and closed canopy environments to minimize light differences as a potential driver of size-related differences in leaf and twig properties. We hypothesized that (H1) gas-exchange rates, tissue N concentration and leaf mass per unit area (LMA) would vary with tree size in a pattern reflecting declining function in taller trees, yet maintaining (H2) bivariate trait relations, common among species as characterized by the leaf economics spectrum. Taking these two ideas together yielded a third, integrated hypothesis that (H3) nitrogen (N) content and gas-exchange rates should decrease monotonically with tree size and LMA should increase. We observed increasing LMA and decreasing leaf and twig R_d with increasing size, which matched predictions from H1 and H3. However, opposite to our predictions, leaf and twig N generally increased with size, and thus had inverse relations with respiration, rather than the predicted positive relations. Two exceptions were area-based leaf N of *Prunus serotina* Ehrh. in gaps and mass-based leaf N of *Quercus ellipsoidalis* E. J. Hill in gaps, both of which showed qualitatively hump-shaped patterns. Finally, we observed hump-shaped relationships between photosynthetic capacity and tree height, not mirroring any of the other traits, except in the two cases highlighted above. Bivariate trait relations were weak intra-specifically, but were generally significant and positive for area-based traits using the pooled dataset. Results suggest that different traits vary with tree size in different ways that are not consistent with a universal shift towards a lower 'return on investment' strategy. Instead, species traits vary with size in patterns that likely reflect complex variation in water, light, nitrogen and carbon availability, storage and use.

Keywords: *Acer rubrum*, dark respiration, LMA, nitrogen, ontogeny, photosynthetic capacity, *Prunus serotina*, *Quercus ellipsoidalis*

Introduction

For a variety of reasons primarily associated with changes in form, it has been shown that metabolic rates vary with organismal size (e.g., Schmidt-Nielson 1984). Size-related changes in metabolic rates have been studied in comparative zoology for over 80 years (Kleiber 1932), but research on the size dependence of physiological traits in plants began relatively recently (e.g., Cregg et al. 1989). In general, there are both theoretical and empirical grounds to support the hypothesis of declining function at both the tissue (e.g., leaf tissue or root tissue) and whole-plant scale as plants grow larger (Yoder et al. 1994, Gower et al. 1996, Ryan et al. 1997, Bond 2000, Koch et al. 2004, Reich et al. 2006), although such results are

not always consistent. For woody plants, such variation could exist at the whole-plant scale due to variation in proportions of tissues even if tissue-level traits were static. For example, the ratio of photosynthetic to respiratory tissues has been shown to decline in larger trees (Gerrish 1990, Delagrange et al. 2004, Machado and Reich 2006), causing an increased respiratory demand with size even if tissue-level traits such as leaf respiration remain static throughout all life stages. Alternatively, a variation could exist at the tissue scale but not at the whole-plant scale if tissue-level variation was offset by changes in the distribution of tissue types.

Variation among taxa in leaf traits has been posited to result from selection for coordinated traits that result in every given taxon being located someplace along a strategy continuum from slow to fast return on investment that is associated with the leaf economics spectrum (Reich et al. 1997, Wright et al. 2004, Falster et al. 2012,). Species with long-lived leaves produce a greater lifetime return in carbon fixed per unit nutrient (carbon, nitrogen (N) or phosphorus) invested relative to species with short-lived leaves (Westoby et al. 2000), which supports the long-standing hypothesis about the benefits of an extended leaf lifespan (Chabot and Hicks 1982). This has been well-supported among species within and across sites (Reich et al. 1997, Westoby et al. 2000, Wright et al. 2004), and although some aspects of these patterns are also observed among similar-sized individuals within a species (Williams et al. 1989, Reich et al. 1991, Falster et al. 2012), whether these multi-trait patterns also generally reflect variation among individuals of differing size within a species is not as clear.

Many studies have demonstrated changes in leaf traits with increasing tree size consistent with the idea that mass-based leaf function declines with size (Fredericksen et al. 1996, Kolb and Stone 2000, Niinemets 2002, Thomas and Winner 2002), including declines in photosynthetic rates at the upper bounds of tree size (Yoder et al. 1994, Bond 2000, Koch et al. 2004), although not all species measured have followed the same pattern (Thomas 2010). Stomatal conductance has been shown to decline in taller trees due to hydraulic limitation, which may reduce intercellular CO_2 concentrations within leaves and result in declines in assimilation (Grulke and Miller 1994, Yoder et al. 1994, Fredericksen et al. 1996, Hubbard et al. 1999). Nonetheless, although several studies demonstrate concurrent declines in foliar stomatal conductance and photosynthetic rates with increasing tree size, the observed decline in stomatal conductance is not always sufficient to explain the reduction in photosynthesis (Niinemets 2002). Even at a constant intercellular CO_2 concentration, size-related declines in photosynthesis have been reported (Miller et al. 1991, Grulke and Miller 1994), indicating that decreases in stomatal conductance are not necessarily the cause of reduced photosynthetic rates in taller trees. However, mesophyll conductance has been shown to mirror stomatal conductance in tall trees, increasing

diffusive resistance for CO_2 within leaves and causing declines in the CO_2 concentration from the intercellular space to the site of carboxylation (Han 2011), which could potentially explain size-related declines in photosynthesis more sufficiently.

Changes in leaf morphology and chemistry with increasing tree size may also lead to declining assimilation rates. Independent of light environment, leaves of taller trees often exhibit a higher dry mass per unit leaf area (Niinemets and Kull 1995, Niinemets 1997b, Rijkers et al. 2000, Cavaleri et al. 2010, Thomas 2010); this has been attributed to plastic responses and selection pressures related to differences in ambient light conditions, wind exposure and physical abrasion (Thomas and Winner 2002). Each of these pressures is expected to increase lignification in leaves in taller trees (Niinemets 1997b, 2002), which may increase tolerance of low water potentials (Niklas 1991, Niinemets et al. 2001). However, these compounds may dilute the proportion of N in relation to carbon in foliage, causing a negative association often observed between leaf N concentration (herein always used to express the mass-based measure of leaf N) and tree height (Schoettle 1994, Gower et al. 1996, Niinemets 1997a). These declines in leaf N concentrations may reflect a similar decline in the production of photosynthetic compounds in foliage of taller trees, leading to reduced photosynthetic rates. In addition to diluting the N concentration in leaves, the increased thickness and/or density and amounts of lignin and carbohydrates may also increase CO_2 resistance from intercellular air space to carboxylation sites in the chloroplasts (Parkhurst 1994, Hanba et al. 1999). Therefore, leaves of tall trees may have reduced levels of CO_2 within their chloroplasts relative to smaller trees.

Although the importance of leaf trait variation with tree size and age is increasingly recognized (Yoder et al. 1994, Bond 2000, Kolb and Stone 2000, Thomas and Winner 2002, Koch et al. 2004, Martinez-Vilalta et al. 2007, Merilo et al. 2009), quantitative characterization of changes in leaf traits throughout multiple life stages is still rare (Rijkers et al. 2000, Niinemets 2002, Thomas 2010). Moreover, whereas changes in leaf traits late in tree ontogeny (or comparisons of seedlings to mature trees) have received considerable attention, studies that include intermediate life stages (e.g., large saplings or pole-size trees) are far less common. Given that some leaf trait changes in early and mid-life may differ from those late in life (Grulke and Miller 1994, Fredericksen et al. 1996, Thomas and Winner 2002, Thomas 2010) both in pattern and in causal agents, it is important to improve our understanding in this area. Furthermore, many models of forest ecosystem dynamics such as those derived from the JABOWA model (Botkin et al. 1972) assume that the properties of leaves do not vary with tree size, and traits such as photosynthetic rates are input as approximate parameterizations. If the assumption that leaf traits remain static as trees increase in size is untrue, as considerable evidence suggests (Yoder et al. 1994, Bond 2000,

Thomas and Winner 2002, Koch et al. 2004, Thomas 2010), the development of generalized relations of leaf traits to tree size for differing tree functional types (conifers, evergreen angiosperms, deciduous angiosperms) will be required before improved models can be advanced.

Here, we expand on prior studies by focusing on traits, life history stages, species types and conditions less studied in previous research on leaf traits and tree size. Specifically, this included (a) a wider range of leaf traits than assessed in many prior studies, including size, leaf mass per unit area (LMA), photosynthetic capacity, dark respiration and nutrient content, (b) twig traits (respiration, chemistry) that have rarely if ever been reported in relation to tree size, (c) a focus on the continuous transition from juvenile to reproductive middle age (less studied than old age or the two-stage contrast between seedling and adult), (d) an evaluation of both 'sun' and 'shade' plants to better address the role of varying light environment among tree sizes and (e) an assessment of whether changes in multiple traits with tree size are consistent with bivariate trait relations common among species as characterized by the leaf economics spectrum (Reich et al. 1997, Wright et al. 2004).

We measured the above chemical, structural and gas-exchange data in three common temperate deciduous forest tree species (*Quercus ellipsoidalis* E. J. Hill, *Populus tremuloides* Michx. and *Prunus serotina* Ehrh.) in east-central Minnesota, USA. In order to determine whether size-related changes occur independent of light environment, we measured seedlings, saplings and mature trees growing in both dense and sparse tree patches, reflecting relatively closed and open canopy conditions (i.e., 'shade' and 'sun' conditions). The following questions were addressed: (i) Do morphological traits, carbon flux rates and N content of leaves and twigs vary with increased tree height? (ii) Are size-related changes similar or different for different traits, such as for respiration and photosynthesis or for leaves vs. twigs? (iii) Are bivariate relations between traits similar when viewed interspecifically and intra-specifically? We hypothesized that (H1) gas-exchange rates and associated functional traits such as tissue N concentration and LMA would vary with tree size in a pattern reflecting declining function in taller trees, yet (H2) relations among trait pairs would be consistent with patterns documented in the leaf economics spectrum (Wright et al. 2004). Taking these two ideas together yielded a third, integrated hypothesis that (H3) N content and both mass- and N-based photosynthesis and respiration should decrease monotonically with tree size and leaf mass per area should increase.

Materials and methods

Field site and species description

This study was conducted in August 2007 at Cedar Creek Ecosystem Science Reserve (CCESR) in east-central Minnesota, USA (45°24′N, 93°12′W). The CCESR is located on the Anoka Sandplain, a glacial outwash area characterized by coarse textured soil low in N (Pastor et al. 1987). This area is located in the transition zone between the central grasslands and the mixed deciduous forest of eastern North America. Measurements took place in plots that have been part of an ongoing study on the effect of fire frequency on oak savannas. We measured in plots that have not been burned since the study began in 1964; according to aerial photos, the site was predominantly inhabited by woodland vegetation with scattered grass community patches prior to the study (Dijkstra et al. 2006). Cedar Creek's woodland/savanna habitats contain 10 tree species, most commonly northern pin oak (*Quercus ellipsoidalis* E. J. Hill) and bur oak (*Quercus macrocarpa* Michx.), with species such as quaking aspen (*Populus tremuloides* Michx.), black cherry (*Prunus serotina* Ehrh.) and red maple (*Acer rubrum* L.) moderately abundant (Peterson and Reich 2001). We chose to study red maple, northern pin oak and black cherry because they were commonly found growing in both relatively open canopy (called gap hereafter) and closed canopy neighborhoods across a wide range of tree size. Red maple is classified as shade tolerant, while northern pin oak is mid-tolerant and black cherry is the least tolerant of these three species (Baker 1949). Average shade tolerance values given by Niinemets and Valladares (2006) are as follows: *A. rubrum*, 3.44; *Quercus velutina* Lamb. (thought to be closely related to *Q. ellipsoidalis*), 2.74; *P. serotina*, 2.46. Maximum height values given by King et al. (2006) are 34 m for *A. rubrum*, 30 m for *Q. velutina* (see above) and 37 m for *P. serotina*.

We determined the height of individuals ranging in size from small seedling to reproductive age growing in an intact forest. Height of saplings <2.0 m tall was measured as the vertical distance between the stem base and the base of the highest live meristem. For individuals taller than 2 m, the height was measured to the highest live leaf. To measure height, we used a folding wooden ruler for plants <2 m tall, and an expandable measuring pole for taller individuals. For each species, 4–5 individuals per size class (<0.5, 0.5–1.5, 1.5–3.0, 3.0–6.0, 6.0–10.0 and >10.0 m) were sampled in both gap (n = 30) and closed canopy (n = 24) microhabitats to enable the evaluation of possible light differences as a potential driver of size-related differences in leaf and twig properties. Individuals were chosen that were found growing close to one another in dense or sparse tree patches to reduce the amount of environmental heterogeneity (i.e., soil moisture, soil nutrients, etc.) in the measurements. Sampling occurred over the course of 4 weeks to control for seasonal variation in traits. Representative leaf and twig samples were collected from the upper third of the canopy of trees to reduce differences in incident light. We measured fully expanded leaves with minimal damage from physical abrasion and herbivory, and only current-year twig growth from the apex of branches was measured. The number of leaves per twig was relatively similar across species, averaging six leaves

per twig for *A. rubrum* and *Q. ellipsoidalis* and seven leaves per twig for *P. serotina*.

Measuring light availability

We measured the instantaneous percentage of above-canopy photosynthetic photon flux density (%PPFD) above each tree using paired quantum sensors (LI-190s, Li-Cor Inc., Lincoln, NE, USA) attached to data loggers (LI-1000, Li-Cor Inc.). This method provides a reliable and accurate estimate of average canopy openness differences and of integrated quantum flux density (Machado and Reich 1999) and was chosen for practical reasons due to the extended length of the cable on the sensor, which allowed it to be extended into the forest canopy to obtain readings above tall trees. Light was measured on three occasions during the week prior to photosynthetic measurements, with the sensor placed above leaves and twigs that were later used for gas-exchange measurements. Each below-canopy measurement was an average of five successive 1 s light readings, which were referenced to an above-canopy measurement made in a large, nearby clearcut. Measurements were made on overcast days in the early morning to avoid the influence of direct sunlight.

Measuring functional traits

Light-saturated photosynthesis (A_{max}) was measured on attached foliage of juvenile trees using an LI-6400 (Li-Cor Inc.). For mature trees whose leaves could not be reached with a ladder, pole pruners were used to detach branches of at least 1 cm in diameter, which were immediately recut under water and used for photosynthesis measurements. Leaves on each branch were measured directly following removal from the tree, ensuring that the measurement was made within 10 min of detachment. Prior studies indicate that leaf gas-exchange rates are not affected by the detachment of branches (Koike 1986, Yoder et al. 1994, Thomas and Bazzaz 1999, Iio et al. 2004, 2009), and we confirmed this by measuring a subset of leaves on juvenile and pole-sized trees prior to and following detachment (K.M. Sendall, unpublished data). However, it has been noted that stomata are slow to respond to a changed environment, sometimes taking over 30 min to acclimate. This should not be a concern in trees measured in gaps, where ambient light levels were similar to chamber light levels, but it is possible that photosynthetic readings in closed canopy conditions reflect stomatal opening of the ambient light environment rather than the chamber environment. Environmental conditions were controlled within the leaf cuvette as follows: CO_2 concentration of 380 µmol mol^{-1}, 45–65% relative humidity and block temperature of 25 °C. Irradiance was held at 1500 µmol m^{-2} s^{-1} using the LI-6400 LED light source for A_{max}, and at 0 µmol m^{-2} s^{-1} for R_d.

Leaf and twig dark respiration rates (R_d) were measured using samples that were collected at the same time each day (between 08.00 and 09.30 h) to control for potential diurnal variation in leaf carbohydrate status. Immediately following detachment, samples were placed in zip-closure plastic bags with moistened paper towels and stored in a darkened chamber at 10 °C. In a laboratory, sample bags were transferred to a second darkened chamber at 25 °C for 30 min prior to R_d measurements. Measurements were completed on the same day as sampling using an LI-6400 with the conifer chamber attachment. Prior research indicates that respiration rates are similar for detached and attached leaves for up to 6 h following cutting (Mitchell et al. 1999, Lee et al. 2005, Machado and Reich 2006). Leaf and twig samples were placed within the conifer chamber, with environmental conditions controlled as above. Samples were kept inside the chamber for ~10 min, and measurements were recorded only after readings had stabilized. We chose to measure R_d using detached leaf and twig samples so that larger amounts of tissue could be used. This led to larger R_d readings for each sample, which helped to minimize measurement error known to be associated with low gas-exchange rates measured with the LI-6400.

Following gas-exchange measurements, leaves were scanned and the projected area was determined using the image-processing software ImageJ (Abramoff et al. 2004). The basal diameter (i.e., the base of the current year's growth) and the length of each twig segment were measured. All samples were oven-dried at 70 °C for at least 72 h to determine dry leaf mass for calculation of LMA (g cm^{-2}) and twig dry mass. Samples were ground and analyzed for N concentrations (ECS 4010 CHNSO Analyzer, Costech Analytical Technologies Inc., Valencia, CA, USA).

Statistical analysis

Analyses for all relationships were based on log–log transformed data, in order to better meet assumptions of the models and to reduce patterns in the residuals. For all data across species and habitats (including traits, light and height), the logarithmic values were either normal or better approximated normal than without transformation. To test for potential effects of light availability on observed relationships, we conducted multiple linear and polynomial regressions for each trait × species × habitat combination including %PPFD as a covariate. In cases where %PPFD was significant in the multiple regression model, we also ran backward stepwise regression models (using Akaike information criterion in model selection) including %PPFD, height, height^2 (when applicable) and interaction terms to identify which variables were the stronger predictors of trait variation. Despite a correlation between height and %PPDF, this was modest and hence the variance inflation factor (always <1.5) was far below any level considered to be of concern (O'Brien 2007).

In order to more directly assess the effect of tree height, all traits were fit with simple linear and polynomial functions

based on the findings of Thomas (2010), who showed that many traits follow unimodal, hump-shaped (rather than linear) patterns with tree size. Fits of both model types were compared and those which seemed most appropriate, based on a combination of R²-value, significance of parameter terms involving height and the patterns of residuals, was selected and presented in the paper. Traits were also fit using basal diameter as the independent variable, with results generally consistent with fits using height (see Appendix Figures S2–S7 available as Supplementary Data at *Tree Physiology* Online). This is not surprising as for the range of sizes sampled, there was a linear allometric relationship between diameter and height (see Appendix Figure S1 available as Supplementary Data at *Tree Physiology* Online). Where non-linear trends (of log–log data) were observed, in the cases of leaf N and photosynthetic rates, we performed monotonicity tests to determine whether the significant polynomial fits resulted from trait maxima at intermediate heights, rather than non-linear, but monotonically increasing or decreasing patterns (Murtaugh 2003). This test uses the best-fitting monotone regression as a null model, which is a step function that either increases or decreases monotonically. This regression was computed first and the residuals were resampled with replacement using bootstrapping and added to the monotone regression function. A polynomial regression was then repeatedly fit to the resampled null dataset. The second-order coefficient of each of these fits was compared with the second-order coefficient from the original polynomial fit, and the *P* value was calculated as the proportion of coefficients from the resampled data of greater absolute value than the original coefficient. In cases where the original second-order coefficient was not significant, the *P* value for the test of the second-order polynomial term is reported.

To further assess whether declines in R_d could be explained by tree size rather than N, we ran a stepwise backward regression using %PPFD, N_{mass} and height as factors and found that only height was significant. Pearson correlation matrices were used to characterize bivariate trait correlations between pooled traits (all light habitats combined). These analyses revealed that the most strongly correlated traits were LMA in relation to area-based leaf traits (N_{area}, A_{area} and $R_{d_{area}}$). Based on these results, mixed model analyses including species and species interactions were run for each trait–trait relationship. All statistical analyses were conducted in JMP statistical analysis software (JMP 10.0.1, SAS Institute, Cary, NC, USA) and R v. 2.15.2 (R Development Core Team 2008).

Results

%PPFD vs. tree size as a predictor of trait patterns

If traits such as photosynthesis, N content and leaf mass per unit area vary with tree size (Carswell et al. 2000, Rijkers et al.

2000, Niinemets 2002, Kenzo et al. 2006), it is possible that this is due in part to variation in light availability, if leaves sampled from seemingly similar canopy positions are at different (and most likely higher) light levels on taller trees. It is therefore of interest to know both whether leaf traits vary with tree size (regardless of causes) and whether this is due to size per se, to co-variation with light environment, or both. To evaluate this, we measured the instantaneous percentage of above-canopy %PPFD above each tree (Figure 1). In our survey, %PPFD was significantly positively correlated with height in *A. rubrum* (both gap and closed canopy groups) and in *P. serotina* trees in gaps. Light was moderately correlated with height ($P < 0.10$) in *P. serotina* trees in closed canopy microhabitats, but there was no correlation in *Q. ellipsoidalis*. However, these gradients in light were relatively modest across the gradient of tree size, especially for *P. serotina*.

As described below, many tissue traits were significantly related to tree height; thus, it is important to clarify whether these relationships were due in part to co-variation with %PPFD and height. To determine this, we conducted multiple regressions for each trait using %PPFD, height and their two-way interaction as explanatory variables (Table 1). The results of these regressions led us to conclude that most of the relationships of tissue traits with tree height are only very modestly or not at all a consequence of co-variation in light and that there are strong effects of tree height once light is accounted for. For example, the strongest light effect involved LMA, which was significantly affected by %PPFD in *A. rubrum* trees in both gap and closed canopy microhabitats and in *P. serotina* in gaps (where the relationships between %PPFD and height were strongest and thus of most concern). Multiple regression analyses of the relationship between LMA and both height and %PPFD using pooled data (gap and closed canopy) for each species indicate that height is still significant (positively) for all three species once light is accounted for. For other traits, such as leaf N content and most gas-exchange rates, %PPFD was not significant in models that include tree height, or was not retained in stepwise regression models that identified tree height as the sole significant variable. In the remaining presentation of results we do not consider further this co-variation with light, but we do briefly revisit this in the discussion.

Leaf morphology and chemistry

The LMA increased linearly with height (on a log–log scale) for all three species in both gap and closed canopy groups, and was generally 20–30% higher in the tallest trees compared with seedlings (Table 1; Figure 1). The LMA is affected by the area and mass of individual leaves, both of which also increased linearly with height (on a log–log scale) for all three species in closed canopy groups (Figure 2). In gaps, both the area and mass of individual leaves increased in *A. rubrum*, leaf mass increased in *Q. ellipsoidalis*, and no trends were observed
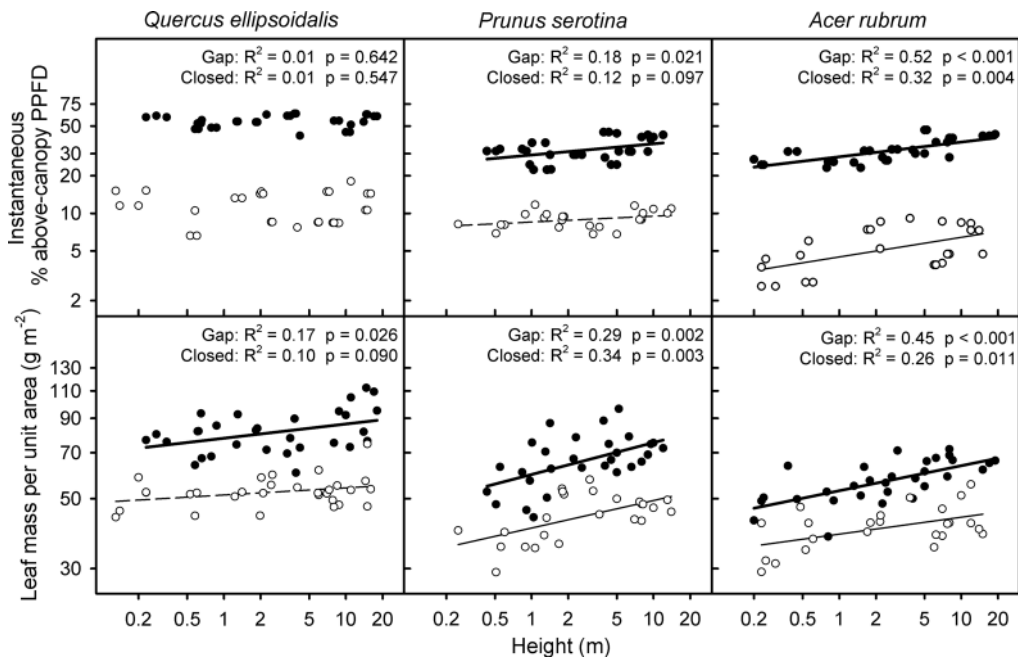
Figure 1. Relationships between instantaneous percent above-canopy %PPFD and tree height, and LMA and tree height for three temperate deciduous tree species; lines are linear regressions fitted to the log–log transformed data. Closed circles and thicker lines represent measurements made in canopy gaps; open circles and thinner lines represent measurements from the closed canopy. Solid lines indicate regressions significant at a P value <0.05; dashed lines at a P value of <0.10.

in *P. serotina*. Similar to the results for LMA, area-based leaf N content (N_{area}) increased continuously with height in all species and groups, with the exception of *P. serotina* in gaps where a hump-shaped relationship was observed (Figure 3). Mass-based N content (N_{mass}) increased continuously with height in both gap and closed canopy groups for *A. rubrum* and in *Q. ellipsoidalis* in closed canopy. No pattern was observed for *P. serotina*, and N_{mass} in *Q. ellipsoidalis* in gaps showed a hump-shaped relationship with tree height (Figure 3). In both cases where the hump-shaped relationships between leaf N and tree height were best explained by polynomial functions, significant second-order terms were observed, but monotonicity tests were not significant (Table 2). This indicates that there is strong evidence for non-linearity, but not for a distinct maximum, which is difficult to show statistically unless sample sizes are quite high.

Leaf gas-exchange

Area- and mass-based photosynthetic rates (A_{area} and A_{mass}, respectively) both varied significantly with height in both *A. rubrum* gap and closed canopy groups, with trait relationships best explained using polynomial functions with significant second-order terms in all cases (Table 1; Figure 4). *Quercus ellipsoidalis* and *P. serotina* showed weaker but qualitatively similar patterns in A_{area} and A_{mass}. The ratio of photosynthesis to dark respiration (A_{max}/R_d) showed similar responses across species with the strongest patterns observed in all *A. rubrum* groups, a significant trend in the *Q. ellipsoidalis* gap data, but no

significant pattern for *P. serotina*. Photosynthesis expressed per unit N (A_{max}/N) showed a marginally significant height relationship and second-order polynomial term for the *A. rubrum* gap and *P. serotina* closed canopy groups. While polynomial functions best explained photosynthetic traits of the three species, monotonicity tests were generally not significant (Table 2), again suggesting non-linearity, but not trait maxima at intermediate heights. Only A_{mass} of *Q. ellipsoidalis* in gaps showed a marginally significant monotonicity result ($P = 0.089$).

Both area- and mass-based leaf dark respiration rates ($R_{d_{area}}$ and $R_{d_{mass}}$, respectively) declined with height in most species groups (Figure 5). These declines were generally between 20 and 35% in magnitude, though in a few cases, declines of up to 50% were observed. Leaf dark respiration expressed per unit N (R_d/N) followed a similar pattern, exhibiting significant linear declines with height in all species groups, except for *P. serotina* in gaps where the decline was marginally significant.

Twig traits

Basal twig diameter of current-year growth at the bud scars showed a linearly increasing allometric relationship with height in *Q. ellipsoidalis* and *A. rubrum* gap and closed canopy groups, but did not vary in *P. serotina* (Figure 6). Twig N_{mass} also increased linearly with height in *Q. ellipsoidalis* gaps and *A. rubrum* closed canopy data, and showed a marginal increase in *P. serotina* gaps. However, twig $R_{d_{mass}}$ declined with height in *A. rubrum* in closed canopy, but did not vary in any other groups. Twig R_d/N declined in *Q. ellipsoidalis* in gaps and *A. rubrum* in closed canopy.

Table 1. Results of multiple regression analyses including %PPFD in addition to height as predictors of leaf and twig traits examined. All data were log transformed prior to analysis. In all cases, %PPFD was included as an additive covariate: for variables described by linear relationships, log $X = a \times \log(H) + b \times \log(P)$ was fitted (where X is the variable in question, H is the tree height and P is %PPFD); for variables described by a polynomial relationship, log $X = a \times \log(H) + b \times \log(H^2) + c \times \log(P)$ was fitted. $P < 0.05$ are indicated in bold; $P < 0.10$ are italicized.

| Variable | Term | Quercus ellipsoidalis | | | | Prunus serotina | | | | Acer rubrum | | | |
| | | Gap | | Understory | | Gap | | Understory | | Gap | | Understory | |
		Param.	P value	Param.	P value	Param.	P value	Param.	P value	Param.	P value	Param.	P value
LMA	PPFD	0.085	0.723	−0.038	0.582	0.286	*0.062*	−0.201	0.331	0.253	*0.089*	0.258	**0.001**
	Height	0.045[1]	**0.031**	0.076[2]	0.109	0.076[1]	**0.023**	0.097[1]	**0.002**	0.050[1]	**0.043**	0.013[1]	0.474
Area per leaf	PPFD	0.386	0.314	0.097	0.618	0.007	0.981	0.498	0.189	−0.123	0.792	0.313	0.190
	Height	0.034	0.288	0.136[1]	**0.001**	−0.021	0.725	0.091[1]	*0.085*	0.189[1]	**0.019**	0.093[1]	0.161
Mass per leaf	PPFD	0.549	0.273	0.101	0.661	0.307	0.345	0.365	0.482	0.065	0.901	0.586	*0.063*
	Height	0.093[1]	**0.029**	0.159[1]	**0.001**	0.072	0.297	0.203[1]	**0.008**	0.276[1]	**0.003**	0.107[1]	0.207
N_{area}	PPFD	−0.028	0.913	−0.125	0.215	−0.107	0.542	−0.215	0.482	0.063	0.727	0.262	**0.029**
	Height	−0.095[1]	**<0.001**	0.071[1]	**0.002**	0.156[1]	**<0.001**	0.093[1]	**0.033**	0.140[1]	**<0.001**	0.056[1]	*0.086*
	Height²	NA	NA	NA	NA	−0.136[2]	0.103	NA	NA	NA	NA	NA	NA
N_{mass}	PPFD	−0.005	0.976	−0.078	0.240	−0.493	**0.004**	−0.019	0.944	−0.206	0.105	0.006	0.943
	Height	0.047[1]	**0.004**	0.049[1]	**0.001**	0.100	**0.005**	−0.002	0.957	0.092[1]	**<0.001**	0.046[1]	**0.047**
	Height²	−0.110[1]	**0.002**	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
A_{area}	PPFD	−0.042	0.949	−0.092	0.704	−0.082	0.729	0.379	0.481	0.129	0.488	0.056	0.770
	Height	0.209[1]	**0.041**	0.053	0.328	0.221[1]	**0.031**	−0.014	0.912	0.218[1]	**<0.001**	0.237[1]	**0.007**
	Height²	−0.185[2]	0.102	−0.093	0.287	−0.156	0.222	−0.254[2]	0.107	−0.111[1]	**0.004**	−0.177[2]	*0.090*
A_{mass}	PPFD	−0.119	0.850	−0.030	0.888	−0.542	*0.089*	0.475	0.367	−0.038	0.856	−0.224	0.235
	Height	0.213[1]	**0.032**	0.028	0.663	0.017	0.897	−0.171[1]	0.167	0.130[1]	**<0.001**	0.229[1]	**0.007**
	Height²	−0.266[1]	**0.017**	−0.059	0.423	0.022	0.890	−0.189[2]	0.211	−0.108[1]	**0.014**	−0.188[2]	*0.064*
A/N	PPFD	−0.106	0.879	0.216	0.427	−0.006	0.979	0.492	0.306	0.102	0.566	−0.213	0.211
	Height	0.088	0.405	−0.041	0.504	−0.051	0.622	−0.166[1]	0.142	0.027	0.347	0.169[2]	**0.026**
	Height²	−0.172	0.152	−0.080	0.411	−0.027	0.836	−0.183[2]	0.184	−0.078[1]	**0.028**	−0.162	*0.083*
A/R_d	PPFD	−0.344	0.507	0.099	0.765	−0.322	0.482	0.491	0.309	−0.224	0.450	0.177	0.468
	Height	0.292[1]	**<0.001**	0.119	0.131	0.081	0.675	−0.017	0.874	0.259[1]	**<0.001**	0.364[1]	**0.002**
	Height²	−0.206[1]	**0.021**	−0.123	0.301	0.031	0.897	−0.015	0.902	−0.077[2]	0.185	−0.283[1]	**0.036**
$R_{d\,area}$	PPFD	0.234	0.632	0.116	0.589	0.258	0.496	−0.150	0.784	0.217	0.336	−0.196	0.102
	Height	−0.081[2]	*0.052*	0.077[2]	*0.080*	−0.015	0.853	−0.117[2]	0.129	−0.072[2]	*0.053*	−0.064[1]	*0.060*
$R_{d\,mass}$	PPFD	0.223	0.540	0.201	0.362	−0.018	0.955	0.119	0.828	−0.099	0.678	−0.439	**0.008**
	Height	−0.109[1]	**0.001**	−0.097[1]	**0.034**	−0.073	0.299	0.199[1]	**0.014**	−0.083[1]	**0.039**	−0.074[1]	*0.091*
R_d/N	PPFD	0.338	0.452	0.283	0.283	0.475	0.171	0.133	*0.085*	0.298	0.299	−0.444	**0.024**
	Height	−0.162[1]	**0.001**	−0.146[1]	**0.009**	−0.174[2]	**0.023**	−0.195[1]	**0.014**	−0.195[1]	**0.001**	−0.117[1]	**0.031**
Twig diameter	PPFD	−0.066	0.812	−0.124	0.221	0.197	0.261	0.160	0.624	−0.384	0.154	0.131	0.249
	Height	0.089[1]	**0.001**	0.075[1]	**0.001**	0.007	0.839	0.017	0.700	0.119[1]	**0.009**	0.069[1]	**0.036**
Twig N	PPFD	0.109	0.603	−0.139	0.141	−0.102	0.627	−0.741	**0.002**	0.072	0.721	−0.028	0.716
	Height	0.051[1]	**0.006**	−0.015	0.409	0.087[2]	*0.060*	0.048	0.119	0.060[1]	*0.092*	0.054[1]	**0.021**
Twig R_d	PPFD	0.239	0.433	0.244	*0.051*	−0.054	0.849	−0.375	0.280	0.611	*0.071*	−0.076	0.515
	Height	−0.036	0.158	−0.024	0.322	0.018	0.764	−0.047	0.332	−0.090	0.100	−0.038[2]	0.249
Twig R_d/N	PPFD	0.038	0.924	0.383	**0.012**	0.048	0.839	0.367	0.271	0.443	0.291	−0.047	0.727
	Height	−0.092[1]	**0.008**	−0.009	0.761	−0.069	0.180	−0.095[2]	**0.049**	−0.111	0.108	−0.093[1]	**0.023**

[1]Indicates cases for which the corresponding term without %PPFD included as a covariate is significant (at $P < 0.05$).
[2]Indicates significance at $P < 0.10$.

Trait–trait relationships

The traits that were most strongly correlated for all species in the pooled (per species) datasets were area-based leaf traits (LMA, N_{area}, A_{area} and $R_{d\,area}$) (see Appendix Table S1 available as Supplementary Data at *Tree Physiology* Online). Mass-based leaf traits rarely showed significant correlations with each other or with LMA. Twig traits were also very weakly correlated, with only twig $R_{d\,mass}$ and N_{mass} exhibiting a clear positive relationship in pooled *P. serotina* data. Using pooled data of all species, we observed significant positive relationships between area-based leaf traits (Figure 7). The slope of the relationships between $R_{d\,area}$ vs. N_{area}, LMA vs. N_{area}, and A_{area}
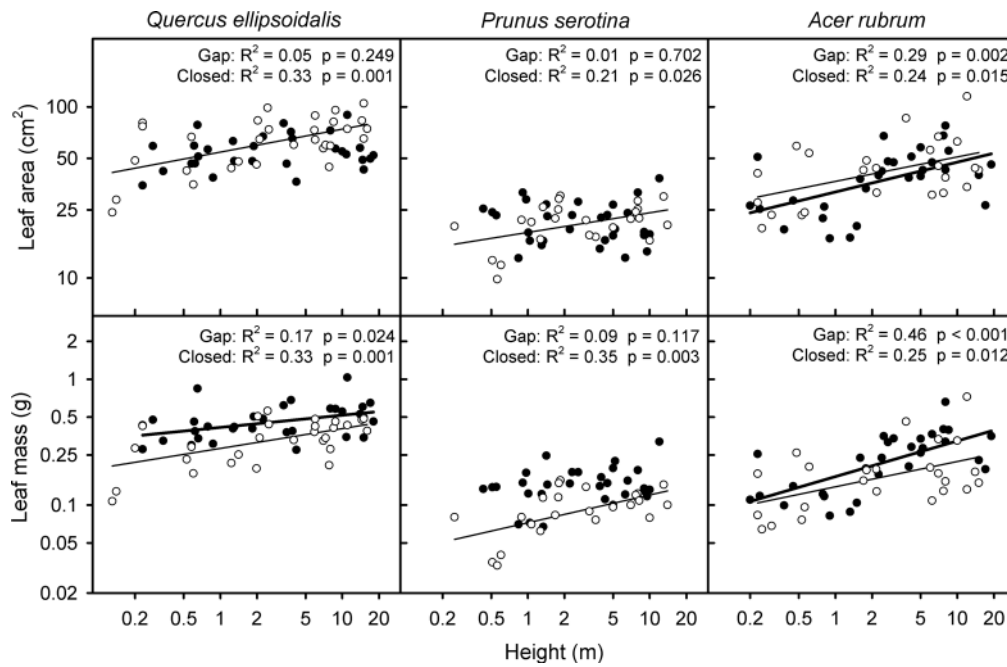
Figure 2. Relationships between area and mass of individual leaves and tree height for three temperate deciduous tree species. Closed circles and thicker lines represent measurements made in canopy gaps; open circles and thinner lines represent measurements from the closed canopy. Lines indicate significant linear regressions fitted to the log–log transformed data at a P value of <0.05.
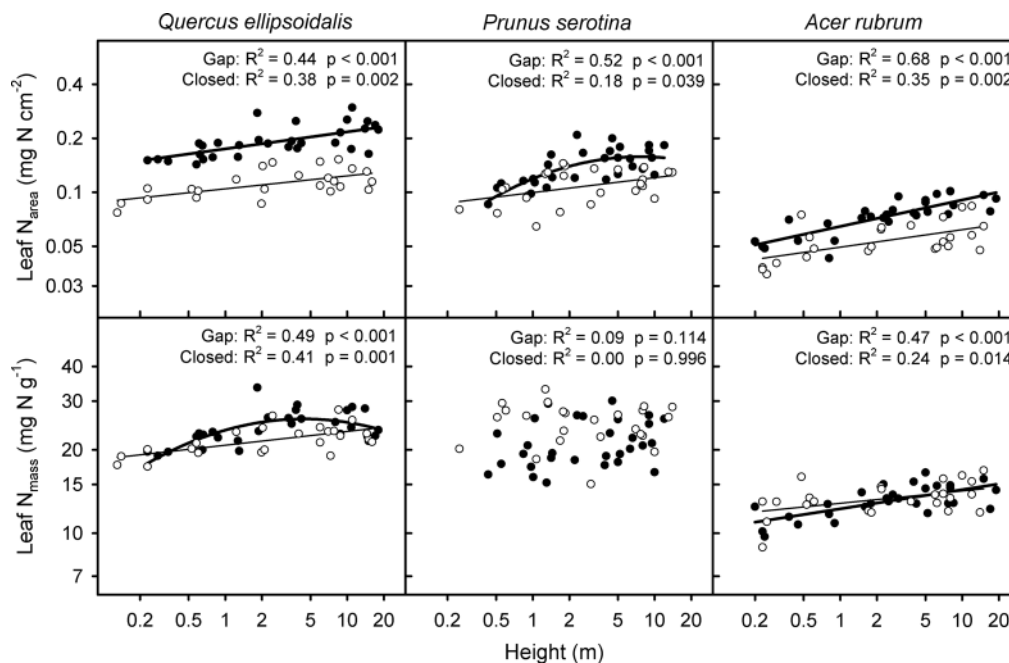

Figure 3. Relationships between leaf N content (expressed on the basis of leaf area (N_{area}) and leaf mass (N_{mass})) and tree height for three temperate deciduous tree species. Closed circles and thicker lines represent measurements made in canopy gaps; open circles and thinner lines represent measurements from the closed canopy. Lines indicate significant linear and second-order polynomial regressions fitted to the log–log transformed data at a P value of <0.05.

vs. $R_{d_{area}}$ was similar for all three species, as evidenced by the non-significant interactions terms in the models (Figure 7b–d). Conversely, the relationship between A_{area} and N_{area} varied among species, with *A. rubrum* exhibiting a steeper increase in A_{area} in response to increased N_{area} relative to the other species (Figure 7a).

Table 2. Results of regression analyses and monotonicity tests of relationships between tree height and leaf N and photosynthetic parameters shown in Figures 3 and 4. Fits of both linear allometric (log $X = a + b \times \log(H)$) and second-order polynomial (log $X = a + b \times \log(H) + c \times \log(H^2)$) equations were compared and the best fit model is presented. P values are shown for both the regression (P(reg)) and Murtaugh (2003) test for monotonicity (P(mono)), and are shown in bold where $P < 0.05$. In cases where the second-order coefficient was not significant in the polynomial model, the P value for the test of the second-order polynomial term is reported as P(mono).

Variable	Parameter	*Quercus ellipsoidalis* Gap	Understory	*Prunus serotina* Gap	Understory	*Acer rubrum* Gap	Understory
N_{area}	a	−0.758	−0.981	−0.896	−1.002	−1.191	−1.306
	b	0.095	0.072	0.145	0.083	0.147	0.097
	c			−0.157			
	R²	0.44	0.38	0.52	0.18	0.68	0.35
	P(reg)	**<0.001**	**0.001**	**<0.001**	**0.040**	**<0.001**	**0.002**
	P(mono)	0.455	0.118	0.205	0.361	0.380	0.221
N_{mass}	a	1.390	1.317	1.298	1.393	1.089	1.107
	b	0.047	0.050	0.056	−0.002	0.069	0.047
	c	−0.102					
	R²	0.49	0.41	0.09	0.00	0.47	0.24
	P(reg)	**<0.001**	**0.002**	0.114	0.933	**<0.001**	**0.014**
	P(mono)	0.309	0.182	0.594	0.977	0.114	0.609
A_{area}	a	0.979	0.748	0.981	0.784	0.787	0.512
	b	0.061	−0.015	0.089	−0.187	0.146	0.114
	c	−0.186	−0.105	−0.174	−0.239	−0.102	−0.186
	R²	0.15	0.08	0.24	0.29	0.79	0.47
	P(reg)	*0.094*	0.373	**0.035**	**0.031**	**<0.001**	**0.001**
	P(mono)	0.319	0.197	0.131	0.120	0.535	0.625
A_{mass}	a	2.113	2.010	2.187	2.174	2.061	1.916
	b	−0.003	−0.017	−0.024	−0.290	0.037	0.064
	c	−0.272	−0.062	−0.095	−0.170	−0.111	−0.152
	R²	0.021	0.03	0.02	0.47	0.42	0.28
	P(reg)	**0.043**	0.641	0.779	**0.002**	**0.001**	**0.030**
	P(mono)	*0.089*	0.357	0.542	0.252	0.499	0.116
A/N	a	0.726	0.675	0.875	0.778	0.957	0.796
	b	−0.051	−0.088	−0.073	−0.280	−0.019	0.246
	c	−0.175	−0.050	−0.028	−0.163	−0.072	−0.128
	R²	0.10	0.12	0.10	0.50	0.17	0.16
	P(reg)	0.238	0.288	0.273	**0.001**	*0.089*	0.153
	P(mono)	0.132	0.571	0.612	0.231	0.468	0.151
A/R_d	a	1.189	1.164	1.195	1.057	1.082	1.158
	b	0.124	0.056	0.021	−0.005	0.170	0.178
	c	−0.215	−0.108	−0.095	0.004	−0.092	−0.312
	R²	0.40	0.12	0.01	0.00	0.62	0.58
	P(reg)	**0.001**	0.250	0.862	0.995	**<0.001**	**<0.001**
	P(mono)	0.281	0.374	0.667	0.975	0.109	0.432

Discussion

Our data demonstrate that a number of important leaf and twig traits such as leaf size, leaf mass per unit area, N content, photosynthetic capacity and dark respiration rates vary significantly with tree size in our three sampled deciduous tree species. These results support our overarching hypothesis that leaf and twig traits vary with increased tree height, but patterns and coordination among traits were less consistent than expected or predicted in specific hypotheses 1–3. Both leaf ($R_{d_{area}}$ and $R_{d_{mass}}$) and twig respiration rates and dark respiration per N content (R_d/N) were lower in larger sized individuals and were best described by linear relationships, which was

consistent with prediction H1 (of declining function with size). Also consistent with H1 was the monotonic increase with tree size in LMA (a morphological trait that reflects investment in leaf structure and defense). In contrast, other traits (e.g., leaf and twig N) expected to decline with size (H1) and be closely positively linked to respiration (H2), increased monotonically or exhibited unimodal relationships with tree size (rejecting H1 and H2). The results for leaf and twig N thus also rejected H3, which assumed parallel linked declines with size for respiration and N. We also observed unimodal relationships between photosynthetic capacity (both A_{area} and A_{mass}) and tree height in the three temperate deciduous species, contrary
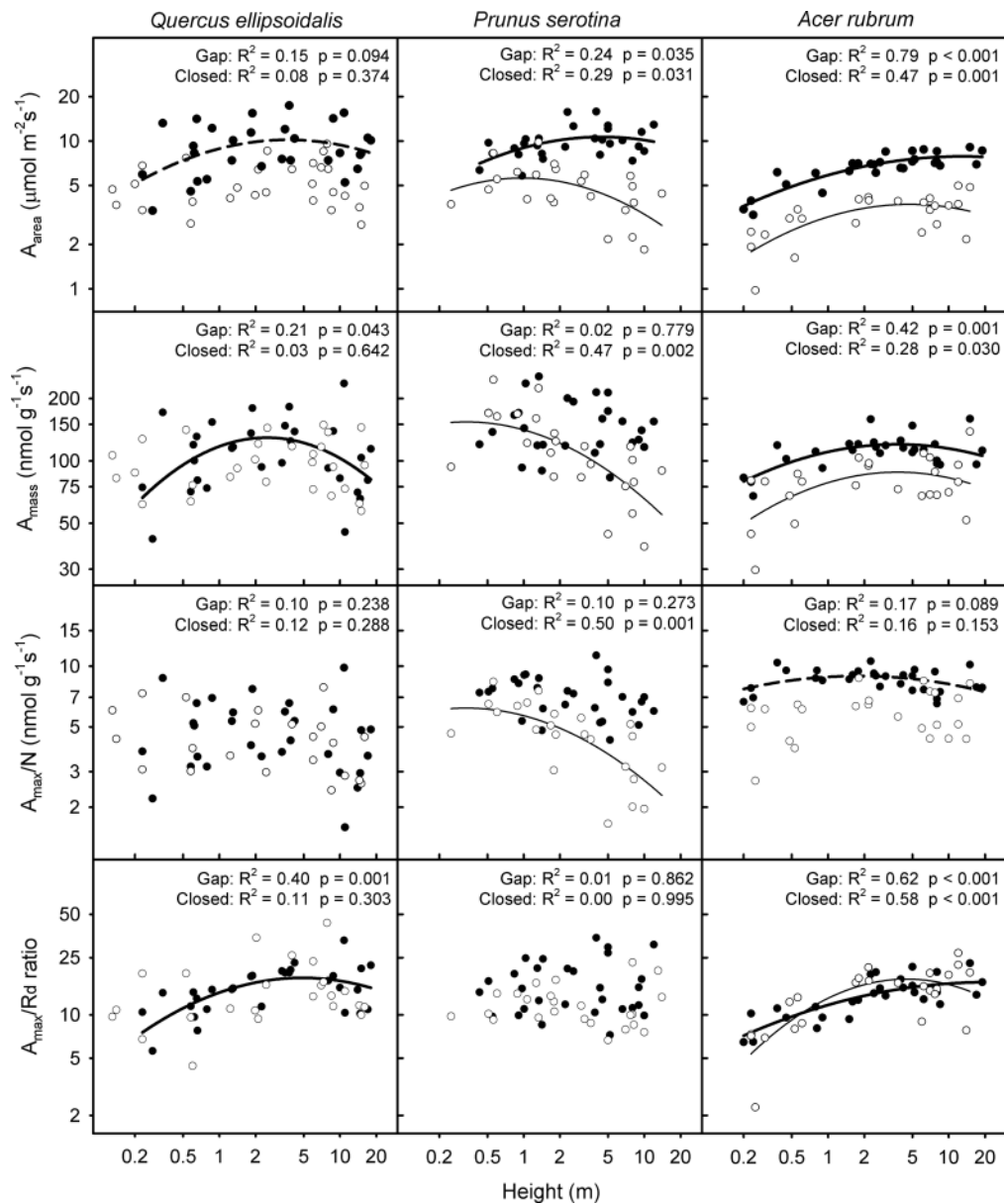
Figure 4. Relationships between photosynthetic capacity (expressed on the basis of leaf area (A_{area}) and leaf mass (A_{mass}), photosynthesis expressed per unit N (A_{max}/N) and the ratio of photosynthesis to dark respiration (A_{max}/R_d)) and tree height for three temperate deciduous tree species; lines are second-order polynomial regressions fitted to the log–log transformed data. Closed circles and thicker lines represent measurements made in canopy gaps; open circles and thinner lines represent measurements from the closed canopy. Solid lines indicate regressions significant at a P value of <0.05; dashed lines at a P value of <0.10.

to expectations of declining function with size (H1). This pattern did, however, mirror N_{area} of *P. serotina* in gaps and N_{mass} of *Q. ellipsoidalis* in gaps, providing some support for H2. In all other cases, the unimodal patterns observed were inconsistent with hypotheses and did not reflect or mirror changes in other traits, such as %N. Although one of the three species (*P. serotina*) showed greater variability in most trait relationships than the others, the trends were generally consistent among species.

While H2 (trait coordination as noted broadly among species) was generally rejected when species and light environments were considered separately, the pooled datasets (across light and species) showed some trends that were consistent with bivariate trait relations common among species as characterized by the leaf economics spectrum (Reich et al. 1997, Wright et al. 2004). For instance, leaves with low $R_{d_{area}}$ also had low A_{area}, while leaves with low N_{area} exhibited low A_{area}, $R_{d_{area}}$ and LMA. In most cases, the strength of these relationships was similar among species. Only in the case of A_{area} vs. N_{area} did we observe species differences in the slope of the relationship, with *A. rubrum* exhibiting a steeper slope relative to *Q. ellipsoidalis* and *P. serotina*.
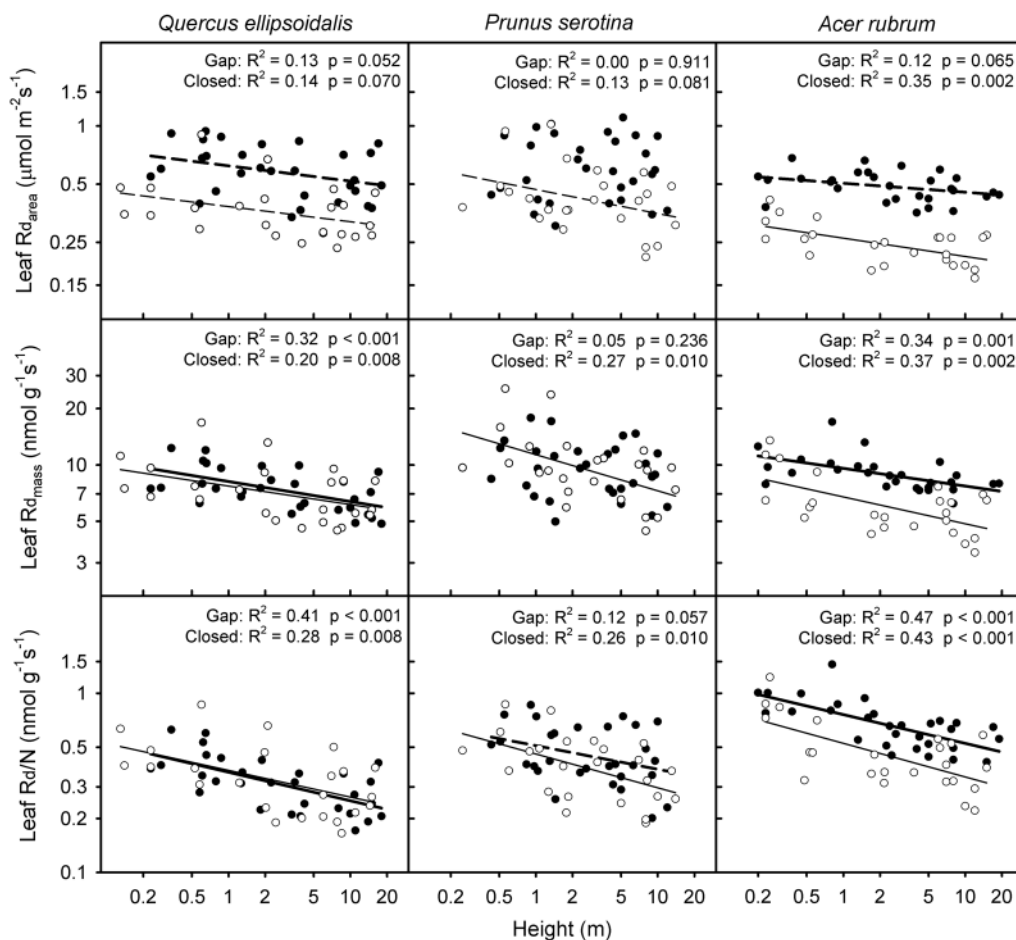
Figure 5. Relationships between dark respiration expressed on the basis of leaf area ($R_{d_{area}}$) and leaf mass ($R_{d_{mass}}$) and tree height, and dark respiration expressed per unit N (R_d/N) and tree height for three temperate deciduous tree species; lines are linear regressions fitted to the log–log transformed data. Closed circles and thicker lines represent measurements made in canopy gaps; open circles and thinner lines represent measurements from the closed canopy. Solid lines indicate regressions significant at a P value of <0.05; dashed lines at a P value of <0.10.

In our study, individuals of all size classes were measured in both gap and closed canopy microhabitats in order to determine whether differences in light availability have an effect on size-related differences in leaf and twig properties. In some other studies, unaccounted co-variation in ambient light may have occurred and could be at least in part responsible for tree-size patterns when light was not assessed or included as a statistical covariate (Carswell et al. 2000, Kenzo et al. 2006). We made tissue measurements on leaves in the upper third of the canopy of trees so as to standardize and maximize their light exposure within each habitat. However, light availability is, not surprisingly, known to be less for juvenile trees even in very large gaps relative to light availability in the upper canopy (Canham et al. 1990, Sipe and Bazzaz 1994), causing a predictable correlation between tree size and incident light. It is well-established that leaf morphology and physiology are influenced by ambient light conditions (Boardman 1977, Givnish 1988), and that variations in light availability are significant predictors of variation in many leaf traits within forest canopies

(Ellsworth and Reich 1993, Kitajima 1994, Rijkers et al. 2000, Kitajima et al. 2005). Therefore, our results (and likely those of many prior studies) are likely to be affected by both tree size as well as ambient light conditions. However, our analyses found that tree size per se was a stronger driver of leaf and twig traits than light differences for similar crown positions of trees differing in size, and that the size effects persisted even once light variation was accounted for. Our analyses that included %PPFD as a covariate indicated strong effects of tree size independent of %PPFD, and in many cases the effect of %PPFD was not significant. Additionally, if light availability were driving apparent ontogenetic patterns, one might expect to find exclusively monotonic relationships between tree height and leaf and twig traits, as opposed to the unimodal patterns we observed in photosynthetic capacity (and also seen in Thomas 2010).

Most research that has aimed at describing ontogenetic variations in leaf photosynthetic traits across a wide range of tree sizes has focused on conifers, and the majority of these studies
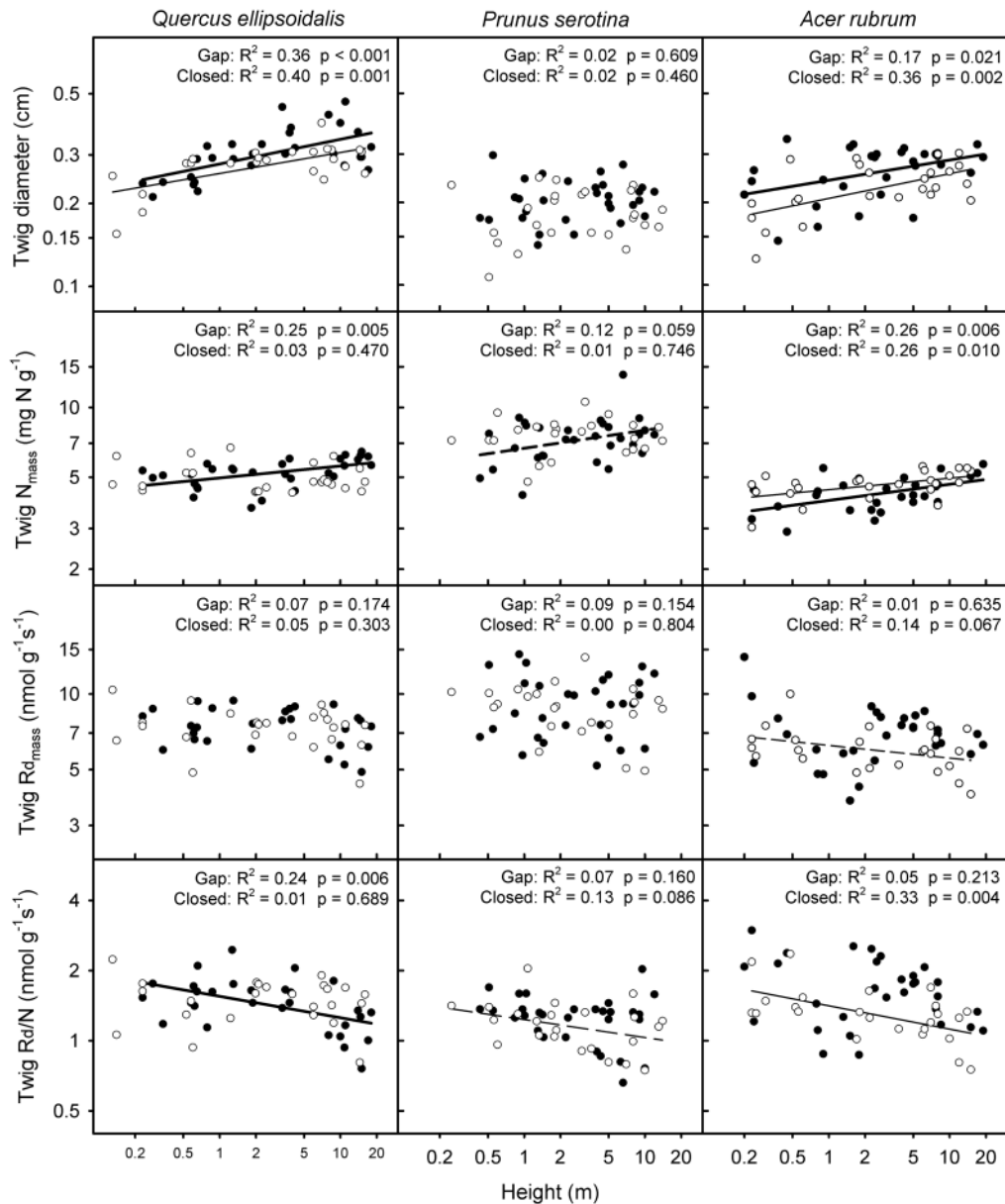
Figure 6. Relationships between twig diameter, twig N content (twig N_{mass}), twig dark respiration (twig $R_{d_{mass}}$) and twig respiration expressed per unit N (twig R_d/N) and tree height for three temperate deciduous tree species; lines are linear regressions fitted to the log–log transformed data. Closed circles and thicker lines represent measurements made in canopy gaps; open circles and thinner lines represent measurements from the closed canopy. Solid lines indicate regressions significant at a P value of <0.05; dashed lines at a P value of <0.10.

have reported declines in photosynthetic rates with tree size and age (Day et al. 2001, Thomas and Winner 2002, Martinez-Vilalta et al. 2007, Greenwood et al. 2008, Merilo et al. 2009, Drake et al. 2010). In contrast, broadleaved species have generally been reported to exhibit higher area-based photosynthetic rates in larger trees (Thomas and Winner 2002, Ishida et al. 2005), while mass-based photosynthesis peaked in sapling-sized individuals (Ishida et al. 2005). Little research has focused on possible causes for ontogenetic increases in photosynthetic rates of broadleaved species, but it has been suggested that roots of larger trees can tap deeper water

resources, allowing them to have higher rates of transpiration and photosynthesis compared with small trees (Bond 2000). Differing somewhat to most of these previously observed patterns, we found that photosynthetic rates (and leaf N in two cases) exhibited qualitatively unimodal relationships as a function of tree size, consistent with the findings of Thomas (2010). Similar to our observations, Thomas (2010) found that the relationship of leaf photosynthetic traits with tree size was better described using polynomial functions than simple linear regressions, and in fact he noted that many prior studies would have benefitted from using this type of fit in their analyses
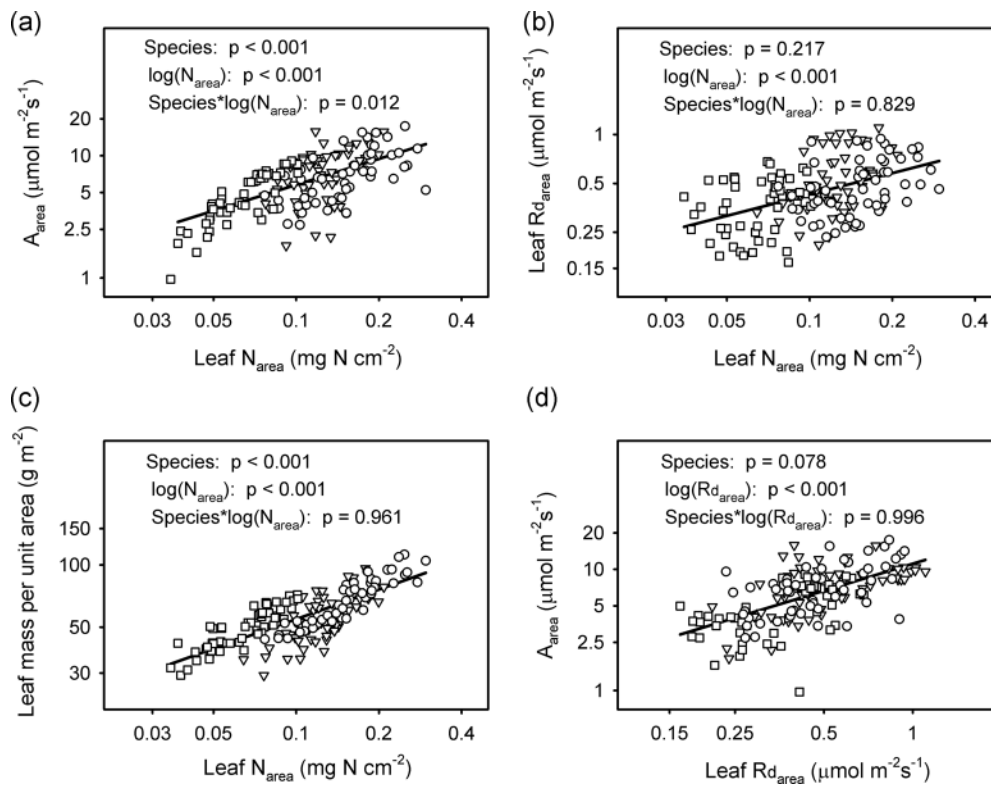
Figure 7. Relationships between (a) area-based photosynthetic rates (A_{area}) vs. area-based leaf N content (N_{area}), (b) area-based leaf dark respiration rates ($R_{d_{area}}$) vs. N_{area}, (c) LMA vs. N_{area} and (d) A_{area} vs. $R_{d_{area}}$ for pooled data from three temperate tree species growing in canopy gaps and under closed canopy conditions. Squares represent measurements from *A. rubrum* individuals, triangles from *P. serotina* and circles from *Q. ellipsoidalis*.

(e.g., Niinemets 2002, Nabeshima and Hiura 2008). However, while the results of Thomas (2010) identified distinct maxima at intermediate heights, our data did not (with the exception of A_{mass} of *Q. ellipsoidalis* in gaps). Instead, they appear to follow non-linear, but monotonically increasing or decreasing patterns. It is possible that our study may not have sampled sufficiently large trees to detect ontogenetic declines at the upper end of the size range, which may help one to explain the lack of trait maxima observed.

Despite the non-linear patterns observed in photosynthetic capacity, leaf N content most often exhibited a size-dependent linear increase across the full range of height. This was unexpected, given the strong correlation that is often observed between photosynthetic rates and leaf N (Gulmon and Chu 1981, Chazdon and Field 1987, Reich et al. 1994). It should be noted, however, that in two of the cases where photosynthetic rates show a qualitatively hump-shaped relationship with height (A_{area}: *P. serotina* gaps, A_{mass}: *Q. ellipsoidalis* gaps), leaf N followed a similar pattern (Figures 3 and 4). The continual increase in leaf N with tree height in all other cases may be due to investment in other properties rather than photosynthetic enzymes in the taller trees, such as chemical defensive compounds (Dominy et al. 2003, Boege 2005).

Wind exposure and physical abrasion are likely to be higher in upper canopy trees than they are in the understory or mid-canopy, favoring more robust leaves with higher LMA in the upper canopy (Thomas and Winner 2002). Higher LMA in taller trees has also been attributed to a reduction in turgor-driven cellular expansion, caused by a decrease in xylem water potential with gravity (Koch et al. 2004, Woodruff et al. 2004, Cavaleri et al. 2010). These might all contribute to greater LMA with tree height, even for leaves in similar light environments. These alternative drivers suggest that prior interpretations of LMA–height patterns may have been too simple (explaining differences in LMA largely to light gradients, i.e., a sun vs. shade leaf explanation) (Harley et al. 1996, Koch et al. 2004, Ambrose et al. 2009); Cavaleri et al. (2010) also concluded that height was a more important predictor than light when explaining the vertical pattern of LMA in a tropical forest.

Proximity of leaves to reproductive structures appears to have negative effects on photosynthetic rates of naturally grown trees (Karlsson et al. 1996, Wheelwright and Logan 2004). Our study was not designed to analyze potential impacts of reproduction on leaf and twig traits, but we can use published literature to make inferences on likely impacts. Declines in carbon uptake in leaves near reproductive structures may be due to nutrient allocation effects, particularly allocation of N to fruits

and seeds (Thomas 2011). For example, Leal and Thomas (2003) reported reduced N content in leaves that developed near reproductive structures in *Pinus strobus* L. trees. However, as stated above, we found an increase in leaf N content across all measured life stages in most cases. If reproductive structures were acting as a local sink for N in our study, we should have more commonly observed a decline in leaf N content in the largest trees measured.

Our hypothesis that leaf and twig respiration rates would decline with tree size was based on empirical evidence showing a positive relationship between total R_d (maintenance plus growth respiration) and relative growth rates (Givnish 1988, Poorter et al. 1990, Walters et al. 1993) and negative relationships of both to plant size (e.g., Reich et al. 2006). Our observations of respiration rates support the hypothesized decline with size, but contradict the positive relationship commonly observed between tissue N concentration and respiration rates (Merino et al. 1982, Waring et al. 1985, Ryan 1991, Reich et al. 2008). Instead, we found that tissue R_d declined with increasing N concentrations in most cases. To further assess whether the decline in R_d could be explained by tree size rather than N, we ran a stepwise backward regression using %PPFD, N_{mass} and height as factors and found that only height was significant (data not shown). This indicates that increased tree height, independent of %PPFD and N_{mass}, is driving the observed decline in tissue respiration rates.

Why were the hypothesized parallel changes in various leaf traits so often unsupported by the evidence? It is possible that increasing N, shifts in allocation of N, increasing LMA and increasing hydraulic limitations (not measured here) with size act to offset each other in terms of effects of net CO_2 exchange rate. All else being equal, increasing N should be associated with increasing photosynthesis and respiration (Gulmon and Chu 1981, Merino et al. 1982, Ryan 1991, Reich et al. 1994), but a lower fraction of that N being allocated to photosynthetic machinery with size could offset that trend. Additionally, as increasing tissue density and/or thickness lead to lower maximum photosynthetic rates due to internal resistance and/or self-shading (Givnish 1988, Parkhurst 1994, Hanba et al. 1999), increasing LMA with size would 'push' net CO_2 exchange rates in an opposite direction than rising tissue N would. For instance, *P. serotina* had the largest response of LMA to size and no variation in mass-based leaf N content with size, and consistent with the ideas above, it showed the most negative relationship of mass-based photosynthesis to size. Furthermore, if hydraulic influences dampen gas-exchange rates in taller trees (Grulke and Miller 1994, Yoder et al. 1994, Fredericksen et al. 1996, Hubbard et al. 1999), this could help explain the plateauing, and sinking, rates of photosynthesis at the tallest tree sizes, despite more linear variation with size in drivers such as N and LMA.

It is less clear why respiration rates of both leaves and twigs should decrease with size, especially in cases where their N

levels continually increase. Our results are perplexing given that a study of *Nothofagus nitida* Ann. K. K. Hofmus trees ranging in size from 0.1 to 7 m in height found respiration rates to be stable, while N increased significantly with tree size (Coopman et al. 2011) and that prior work with temperate saplings from 0.3 to 1.5 m in height showed increasing leaf and twig respiration with height but stable or decreasing tissue N (Machado and Reich 2006). It is possible that a greater fraction of N is allocated to non-metabolic functions with increasing height across a large size range (as in the present study) and/or that respiration in leaves and twigs high in canopies or larger trees is more substrate-limited (i.e., limited by the availability of proteins and total non-structural carbohydrates), but additional research is needed to assess these possibilities. It may also be that size-related shifts in respiration and N are different across a modest size range for small individuals also sampled across a range of low light availability (and thus different growth rate history) (as in Machado and Reich 2006) than across a much larger size range, but at standardized (and higher) light level, as in the current study. However, disentangling the roles of size, age, resource supply and growth rate in controlling intra-specific respiration will require considerable additional research.

In conclusion, leaf and twig traits vary intra-specifically with increased tree height, independent of variation in light availability, but not in the consistent manner associated with a universal shift toward a lower 'return on investment' strategy. In other words, traits did not vary intra-specifically in the coordinated fashion we expected based on the leaf economics spectrum (Reich et al. 1992, Westoby et al. 2002, Wright et al. 2004), nor uniformly reflect a declining tissue function with increased size. Low leaf respiration rates did not correspond with low leaf N content, while photosynthetic rates did not increase in parallel with leaf dark respiration rates. Instead, species traits varied with size in patterns that likely reflect complex variation in water, light, N and carbon availability, storage and use; with consequential impacts on morphology, chemistry and metabolism of leaves and twigs. These conclusions have implications for models of forest composition and ecosystem dynamics, since to our knowledge all such models assume that the properties of leaves do not vary with tree size per se, which from our work and that of others is clearly not true. However, the wide variety of size-related patterns observed across the small number of studies of this type indicates that we remain frustratingly far from being able to develop the generalized size-dependent leaf and twig functions needed to establish general principles and improve vegetation, ecosystem and land surface models.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

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